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The ESTHER database on alpha/beta hydrolase fold proteins - an overview of recent developments

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Abstract

The ESTHER database, dedicated to ESTerases and alpha/beta-Hydrolase Enzymes and Relatives (<https://bioweb.supagro.inra.fr/ESTHER/general?what=index>), offers online access to a continuously updated, sequence-based classification of proteins harboring the alpha/beta hydrolase fold into families and subfamilies. In particular, the database proposes links to the sequences, structures, ligands and huge diversity of functions of these proteins, and to the related literature and other databases. Taking advantage of the promiscuity of enzymatic function, many engineered esterases, lipases, epoxide-hydrolases, haloalkane dehalogenases are used for biotechnological applications. Finding means for detoxifying those protein members that are targeted by insecticides, herbicides, antibiotics, or for reactivating human cholinesterases when inhibited by nerve gas, are still active areas of research. Using or improving the capacity of some enzymes to breakdown plastics with the aim to recycle valuable material and reduce waste is an emerging challenge. Most hydrolases in the superfamily are water-soluble and act on or are inhibited by small organic compounds, yet in a few subfamilies some members interact with other, unrelated proteins to modulate activity or trigger functional partnerships. Recent development in 3D structure prediction brought by AI-based programs now permits analysis of enzymatic mechanisms for a variety of hydrolases with no experimental 3D structure available. Finally, mutations in as many as 34 of the 120 human genes compiled in the database are now linked to genetic diseases, a feature fueling research on early detection, metabolic pathways, pharmacological treatment or enzyme replacement therapy. Here we review those developments in the database that took place over the latest decade and discuss potential new applications and recent and future expected research in the field.

Foreword

Conceiving the present paper, to be included in these proceedings of the 14th International Meeting on Cholinesterases held in Bologna (Italy), prompted us to read again a chapter by a late prominent semiotic professor at the *ca.* one millennium-old university in this city: Umberto Eco. Eco wrote on the impossibility of drawing a map of the Empire at a 1-to-1 scale [1], and in a foreword he acknowledged the priority of this description in a publication by another famous writer, Jorge Luis Borges. After spending so many years curating the ESTHER database, we also found appropriate to cite Borges for his invented translation of an old manuscript containing a humoristic classification of animals [2]. (An excerpt of this classification is included in the database acknowledgment page <https://bioweb.supagro.inrae.fr/ESTHER/general?what=acknowledgement>). Indeed, exhaustivity in databases dedicated to biological molecules and publicly available on the net is also an unreachable goal, since the already overwhelming volume of produced data keeps growing exponentially with time [3]. The diversity of activities performed by the different families of alpha/beta hydrolases clearly fit with the “state of shambles” which some users can feel when connecting to the ESTHER database. However, in remembering the beginnings of our pioneering endeavors we are confident in that the database keeps improving and better meets the needs of the community.

Introduction

The alpha/beta hydrolase fold was first described three decades ago [4] and the ESTHER database, dedicated to proteins sharing this fold, was created three years later in 1995. Since then, the number of enzymatic activities and biological functions found to be associated to members of this superfamily has continually increased. The latest articles presenting the database are now 10 years old [5, 6] while a wealth of discoveries were made since then. For example, the number of subfamilies went up from 148 to 235, and the number of experimentally determined 3D structures increased two fold to now reach 3000. Many reviews have dealt with the superfamily [7-11] and sister

databases dedicated to special focuses on the structural fold or concentrating on a particular activity were launched: the Lipase Engineering Database LED [12] and its update [13], The alpha/beta hydrolase fold 3DM database (ABHDB) [14], the Thioester-active enZYmes database (ThYme) [15], the PHA Depolymerase Engineering Database (DED) [16], the Plastics-Active enZYmes database PAZy [17], database PlasticDB dedicated to microorganisms and proteins linked to plastic biodegradation [18], the Comprehensive Plastics Microbial Biodegradation Database (PMDb) [19], the Carbohydrate-Active enZYme database CAZY [20], the peptidase database MEROPS [21]. All families in ESTHER are cross-referenced with links to some of these databases. A compilation of these links is available in the complete table of families (<https://bioweb.supagro.inra.fr/ESTHER/general?what=overallTable>) (to see the details, open the legend sign). It includes widely used classifications that are not supported by databases on their own such as the Arpigny and Jaeger classification of bacterial lipases and its updates [22, 23] or the classification of fungal feruloyl esterases [24]. Experimental X-ray crystallography data that could not be processed due to the lack of a suitable molecular replacement template can now be solved using templates modeled with AI-based programs, such as AlphaFold or RoseTTAFold [25-27]. The models themselves now provide users with unprecedented capacity to rationalize enzymatic capacity of most enzymes devoid of an experimentally solved 3D structure. The cross-reference links between ESTHER and Uniprot [28] provide indirect access to these models, as they do for experimental 3D structures.

Scope and limits of the ESTHER database

Representative gene	Structural family
SEC23IP	SEC23-interacting protein DDHD domain
DDHD2	DDHD domain. DDHD four residues forming a metal-binding site
FAAH	Fatty acid amide hydrolase Amidase signature (AS) superfamily
PNPLA2	Patatin-like phospholipase domain-containing protein 2, ATGL, Adipose triglyceride lipase, Calcium-independent phospholipase A2-zeta (iPLA2-zeta)
PNPLA4	Patatin-like phospholipase domain-containing protein 4
PNPLA6	Patatin-like phospholipase domain-containing protein 6 (PLPL6), Neuropathy target esterase (NTE), PG39, sws [29]
PNPLA7	Patatin-like phospholipase domain-containing protein 7
PNPLA8	Neuropathy target esterase-related esterase (NRE)
PAFAH1B2	Calcium-independent phospholipase A2-gamma, IPLA22, IPLA2G, BM-043 Acyl transferase/acyl hydrolase/lysophospholipase Patatin
Haemagglutinin-esterase	Platelet-activating factor acetyl hydrolase IB subunit alpha2 SGNH hydrolase superfamily GDSL esterase/lipase
Rhamnogalacturonan acylesterase	SGNH hydrolase superfamily
SEST-like	RhgT-like SGNH hydrolase superfamily, GDSL esterase/lipase
IAH1	Streptomyces scabies esterase-like EstA SGNH hydrolase superfamily, GDSL esterase/lipase
HTRA2	Isoamyl acetate-hydrolyzing esterase 1 homolog SGNH hydrolase superfamily
TPP2	Peptidase S1, PA clan chymotrypsin-like fold
LACTB	Tripeptidyl-peptidase 2, Peptidase_S8_His-AS
Glutaminase	Peptidase S11 S12 family, D-ala carboxypeptidase/transpeptidase, esterase (EstB)
DPP3	GLS Beta-lactamase/transpeptidase-like Glutaminase
Cathepsin C	Dipeptidyl peptidase 3, DPP3, Peptidase family M49 metallopeptidase
CATC	Dipeptidyl peptidase 1, Cathepsin C
	Papain-like cysteine peptidase superfamily

Ancestral alpha/beta hydrolases are essentially water-soluble proteins acting intracellularly or being secreted. Through addition of modules, lids or anchoring motifs to the genuine structural core, some members of the

superfamily gained affinity for lipids or protein-binding/recognition properties enabling formation of oligomers or interaction with functional partners. Some others gained membrane linkage properties and even the capacity of transducing signals intracellularly. However, no example of an alpha/beta hydrolase participating in a macromolecular multi-protein scaffold/assembly has yet been reported.

Proteins harboring the alpha/beta hydrolase fold constitute a single superfamily showing the highest diversity of functions in comparison to other structural folds. The diversity of catalytic mechanisms acquired in the different families has been reviewed previously [10, 11]. The most puzzling new activity discovered recently is the post-translational epimerization of L-Asp into D-Asp during bottromycin biosynthesis by bottromycin epimerase BotH, which does not rely on a catalytic triad [30]. Noteworthy however, there is not a single activity or function entirely performed only by members of the alpha/beta hydrolase fold superfamily. Emblematically, those plant enzymes often defined as “acetylcholinesterases” are in fact GNH esterases, and a role of acetylcholine outside the animal phylum is still actively scrutinized. In the peptidase database MEROPS, only the families of the clan SC of serine peptidases S9, S10, S15, S28, S33, S37 and S82 are alpha/beta hydrolases. All others are not, such as in particular the chymotrypsin family, with its catalytic triad that has a mirror geometry to the one found in some alpha/beta hydrolases. In the bacterial lipase classification, family VIII presents a beta-lactamase fold and family II a patatin fold (SGNH superfamily). The following table (Table 1) provides examples of proteins compared to alpha/beta hydrolases or even wrongly incorporated into the superfamily.

Table 1 : Major families of esterases not harboring the alpha/beta hydrolase fold

Representative gene	Structural family
SEC23IP	SEC23-interacting protein DDHD domain
DDHD2	DDHD domain. DDHD four residues forming a metal-binding site
FAAH	Fatty acid amide hydrolase Amidase signature (AS) superfamily
PNPLA2	Patatin-like phospholipase domain-containing protein 2, ATGL, Adipose triglyceride lipase, Calcium-independent phospholipase A2-zeta (iPLA2-zeta)
PNPLA4	Patatin-like phospholipase domain-containing protein 4
PNPLA6	Patatin-like phospholipase domain-containing protein 6 (PLPL6), Neuropathy target esterase (NTE), PG39, sws [29]
PNPLA7	Patatin-like phospholipase domain-containing protein 7 Neuropathy target esterase-related esterase (NRE)
PNPLA8	Calcium-independent phospholipase A2-gamma, IPLA22, IPLA2G, BM-043 Acyl transferase/acyl hydrolase/lysophospholipase Patatin
PAFAH1B2	Platelet-activating factor acetyl hydrolase IB subunit alpha2 SGNH hydrolase superfamily GDSL esterase/lipase
Haemagglutinin-esterase	SGNH hydrolase superfamily
Rhamnogalacturonan acylesterase	RhgT-like SGNH hydrolase superfamily, GDSL esterase/lipase
SEST-like	Streptomyces scabies esterase-like EstA SGNH hydrolase superfamily, GDSL esterase/lipase
IAH1	Isoamyl acetate-hydrolyzing esterase 1 homolog SGNH hydrolase superfamily
HTRA2	Peptidase S1, PA clan chymotrypsin-like fold
TPP2	Tripeptidyl-peptidase 2, Peptidase_S8_His-AS
LACTB	Peptidase S11 S12 family, D-ala carboxypeptidase/transpeptidase, esterase (EstB)
Glutaminase	GLS Beta-lactamase/transpeptidase-like Glutaminase
DPP3	Dipeptidyl peptidase 3, DPP3, Peptidase family M49 metallopeptidase
Cathepsin C	Dipeptidyl peptidase 1, Cathepsin C
CATC	Papain-like cysteine peptidase superfamily

The following table (Table 2) presents the most recently defined new families created by extracting subfamilies from the large family 6_AlphaBeta_hydrolase, which mostly contained uncharacterized proteins, or by

splitting original families in which a new, separate enzymatic function has recently been described. When a protein has been experimentally characterized and some enzymatic activity or 3D structure is known for this lead sequence, these features enable isolation of an alignment seed and creation of a new family. In most cases this process follows the creation of a specific entry in Interpro database [31].

Table 2 New families in ESTHER.

Representative family	Comment
Tiancimycin-TnmK-Tripeptidase-HIP	Formation of a disulphide bond between the anthraquinone moiety and enediyne. Hip1 (Hydrolase important for pathogenesis-1) from <i>Mycobacterium tuberculosis</i> [32, 33]
BD-FAE	Bifunctional feruloyl and acetyl xylan esterase [34]
BIEst2-lipase-like	<i>Bacillus licheniformis</i> lipase BIEst2 homologues
Abhydrolase_10	The name comes from Pfam database but different from ABHD10
Dieckmann_Cyclase	A conserved thioesterase-like scaffold has evolved to perform a new chemical reaction: heterocyclization
BotH	The bottromycin epimerase BotH family
ABHD8	This family was initially included in the Epoxide_hydrolase family but the active site nucleophile is a Serine in ABHD8
ABHD16	Phosphatidylserine lipase ABHD16A
ABHD11-Acetyl_transferase	Homologues of human ABHD11 and yeast Eat1
T6SS-TLE3	Tle3 phospholipase effector from adherent-invasive bacteria
Chlorophyllase_Plant	Separated from bacterial chlorophyllase/Cutinase
Fungal-D14-Strigolactone-R	Fungal strigolactone receptors are different from those from plants
Mbeg1-like	The name of family Duf_2974 was changed for Mbeg1-like [35]
Crenarchaeota-hydrolase	abH10.01 (uncultured crenarchaeote) in the LED database
Extracel-MCL-phaZ	Extracted from 5_AlphaBeta hydrolase
Polyesterase-MGS0156-like	Extracted from 6_AlphaBeta hydrolase
yjFP_esterase-like	Extracted from the large AlphaBeta hydrolase family
FAE-Bacterial-promiscuous	Extracted from the large AlphaBeta hydrolase family
T6SS-TLE1	The name of family Duf_2235 was changed for T6SS-TLE1
Est-OsmC	Carboxyl Esterases with an OsmC Domain [36]
Extracel-MCL-phaZ	Extracellular medium-chain-length poly(3-hydroxyalkanoate) depolymerase

The most recent developments in research on alpha/beta hydrolases concern both long-standing difficulties such as in vivo reactivation of cholinesterases inhibited by organophosphates (OP) and new functions such as degradation of plastic waste. Below we summarize some of these areas of research, which elicited numerous recent papers and seeded major updates of the database, with new families, new 3D structures and new compounds (substrates, inhibitors, reactivators). Each compound is cross-referenced with PubChem or UniChem databases [37, 38] and classified by type of chemical, which can be a family of reactants or a functional group of the molecule.

Organophosphates and reactivators

OPs, initially designed as warfare agents (nerve gas or nerve agent) or as insecticides, are potent inhibitors of cholinesterases (the prototype of alpha/beta hydrolases) from all animal species. They do so by phosphorylation of the active site serine, thereby forming an inactive “adduct” that is typically not spontaneously reversible in hours-long time intervals, possibly resulting in a lethal outcome. Many other serine hydrolases (*i.e.*, enzymes with a serine as the active site nucleophile) are secondary targets of OPs. A few, which are not alpha/beta hydrolases but patatine-like esterases (*e.g.*, the neurotoxic esterase NTE), are responsible for the delayed neuropathy that can follow OP intoxication. OPs can form adducts in other, totally unrelated proteins [39], and even phosphorylate other residues

than serine [40]. However, attempts to analyze, all serine hydrolases in complex tissues through activity-based protein probe experiments (ABPP) using a nonspecific OP inhibitor probe, resulted in selection of mainly alpha/beta hydrolases [41]. Cyclic OPs derived from natural bacterial compounds, which are less toxic for animals, were recently developed as potential drugs against mycobacteria [42, 43].

Since the discovery, by Wilson and Ginsburg 70 years ago, of 2-PAM/pralidoxim as the first practically efficient reactivator of cholinesterase inhibited by warfare agents or insecticides [44], thousands of preexisting or newly-designed compounds have been tested with the aim of finding new hints generating higher survival rates and fewer deleterious side effects and easy to store and deliver, *i.e.*, more suitable for use in humans. Representatives of reactivators are found in page <https://bioweb.supagro.inrae.fr/ESTHER/reactivatorTable>. Among the series synthesized and described in the articles, only the most active or the lead compounds have been incorporated in the database.

Promiscuity of enzymatic reaction

Many alpha/beta hydrolases are involved in pathways for natural product biosynthesis rather than central metabolism, and as such, they may evolve toward great promiscuity of substrate hydrolysis [45]. The great diversity of reactions performed by enzymes bearing the alpha/beta hydrolase fold, which all originate from a common ancestor, explains how some enzymes can naturally evolve toward catalysis of more than one chemical transformation [11, 46, 47]. Besides classical hydrolytic reactions such as those performed by lipases, esterases, peptidases, or epoxide-hydrolases, in other enzymes similar active sites can perform more peculiar reactions such as dehalogenation, Knoevenagel condensation, Diels-Alder reaction [48], cofactor-independent deoxygenation [49]. For example, *S. laurentii* enzyme Tsr1 is able to perform two different reactions successively: endopeptidyl hydrolysis and epoxide ring opening/macrocyclization [50]. Leaving aside the numerous works where mutations were introduced in order to modify the substrate specificity of enzymes another approach was developed where a versatile metal-chelating inhibitor transformed an esterase into an enantioselective catecholase [51]. Some hydrolases are sufficiently resistant to organic solvent to be able to catalyze the reverse reaction, in which water is a product rather than a substrate. Interestingly, some lipases and esterases can catalyze acyltransferase or transesterification reactions in water. (Again, alpha/beta hydrolases are not the only or best enzymes for performing such reactions: members of the SGNH hydrolase, GDSL lipase or family VIII esterases families can make it as well [52].) Confronting collections of proteins with collections of substrates helped to classify enzymes according to their propensity for hydrolyzing multiple substrates [53], and to develop machine-learning tools to predict promiscuity [54]. The versatility can be exploited by selecting microorganisms that produce enzymes with the desired promiscuity of substrates. Tools are developed to predict the promiscuity capacities of enzyme from 3D models of the active site [53]. Another step consists in adding and enhancing such catalytic activities by protein engineering. High- and ultrahigh-throughput techniques such as droplet microfluidics can rapidly select new enzymes [55].

Enantioselectivity, thermostability and tolerance in organic solvents

Enantioselectivity, thermostability and tolerance in organic solvents are key parameters for enzymes to replace chemical catalyst in order to increase the competitiveness of the corresponding industrial process. Techniques used to engineer tailored enzymes evolved rapidly in recent years and cannot be all cited here. In the case of alpha/beta hydrolases, some queries of the ESTHER database will help collect easily references and enzymes used for this purpose. The following queries can be copied or edited in the AQL query page (<https://bioweb.supagro.inrae.fr/ESTHER/aqlquery>). The following links lead directly to the results page:

```
select p,t,y from p in class Paper, t in p->Title where t like "**enantio*", y in p->Year where y like "2*" order by :y desc
```

[https://bioweb.supagro.inrae.fr/ESTHER/aqlquery?aql=select%20p,t,y%20from%20p%20in%20class%20Paper,%20t%20in%20p%20%2D%3ETitle%20where%20t%20like%20"*enantio*",%20y%20in%20p%20%2D%3EYear%20where%20y%20like%20"2*"%20order%20by%20%3Ay%20desc](https://bioweb.supagro.inrae.fr/ESTHER/aqlquery?aql=select%20p,t,y%20from%20p%20in%20class%20Paper,%20t%20in%20p%20%2D%3ETitle%20where%20t%20like%20)

```
select p,t,y from p in class Paper, t in p->Title where t like "**thermostab*", y in p->Year where y like "2*" order by :y desc
```

[https://bioweb.supagro.inra.fr/ESTHER/aqlquery?aql=select%20p,t,y%20from%20p%20in%20class%20Paper,%20t%20in%20p%20D%3ETitle%20where%20t%20like%20"*thermostab*",%20y%20in%20p%20D%3EYear%20where%20y%20like%20"2*"%20order%20by%20%3Ay%20desc](https://bioweb.supagro.inra.fr/ESTHER/aqlquery?aql=select%20p,t,y%20from%20p%20in%20class%20Paper,%20t%20in%20p%20D%3ETitle%20where%20t%20like%20)

Recent new tools for discovery of substrates and inhibitors specific for orphan enzymes.

The inference of function from a peptidic sequence or 3D structure only is still a challenge [56], and many subfamilies contain “DUF” members (for Domain of Unknown Function) that still await attribution of a biological role. Loss-of-function mutants either experimentally generated or discovered as determinant of naturally occurring genetic diseases lead to cross-link metabolic pathways to specific enzymes and confirm or postulate the specific substrate of a particular enzyme: see <https://bioweb.supagro.inra.fr/ESTHER/disease.table> (in particular ABHD12, ABHD16, DPP9). The more recent activity-based protein profiling (ABPP) strategy is a chemical proteomics approach that utilizes an unspecific inhibitor bearing both a reactive group able to link all serine hydrolases covalently and a fluorescent or affinity tag not interfering with ligand binding. Competition of this traceable inhibitor with compounds from chemical libraries allows one to select inhibitors or substrates specific for one enzyme only [57, 58]. High-resolution liquid chromatography with tandem mass spectrometry (LC-MS-MS) of possible substrates, compared to quantitative trait loci (QTLs) pertinent for their level in biological tissues, gave hints at specific substrates of ABHD1 and ABHD2 [59, 60].

Alpha/beta hydrolases involved in hydrolysis of plant lignocellulose biomass

Microorganisms are able to hydrolyze complex polymers of plant polysaccharides or polyphenols. Interestingly, none of the glycoside hydrolase, glycosyl transferase or polysaccharide lyase families as defined in the CAZy database are alpha/beta hydrolases, while many carbohydrate hydrolase families classified in CAZy are true alpha/beta hydrolases (families CE 1,5,7,10,13,15,19) [20] and as many are not but instead belong to the SGNH superfamily (CE 2,3,6,12,17,20) [61]. There is no alpha/beta hydrolase known to hydrolyze the most abundant polymers of the plant cell walls such as lignin, or structural polysaccharides such as cellulose and chitin, but some are cutin or suberin hydrolases, and some esterases act on ester bonds of compounds that decorate the lignin backbone, thereby preparing the way for glycoside hydrolases able to achieve complete breakdown of the substrate.

The polyester compounds polyhydroxyalkanoates (PHAs, PHB) are produced and degraded by many bacteria, which incorporate them in the form of highly refractive granules and rely on them as both a source of energy and a carbon reserve/store. PHAs are thus considered as “natural” plastics and are copied to generate industrial biodegradable polymers. Similarly, some lipases can catalyze polycondensation or ring opening polymerization reactions [62]. Many alpha/beta hydrolase families contain such enzymes: PolyAspartate-hydrolase, OHBut_olig_hydro_put, PHAZ7_phb_depolymerase, PHB_depolymerase_PhaZ, BD-FAE, Tannase, Esterase_phb, Esterase_phb_PHAZ, FaeC. The complexity of the distribution of these activities can be exemplified in the case of feruloyl esterases, which hydrolyze phenolic groups involved in the cross-linking of arabinoxylan to other polymeric structures. Initially these enzymes were distributed in different sub-classes: Type-A to F, according to their substrate preferences [63]. These classes are now respectively included into the following ESTHER families: Type-A in Lipase_3, Type-B in Esterase_phb (PHB depolymerase), Type-C in Tannase, Type-D in FaeC, Type-E in A85-Feruloyl-Esterase, Type-F in BD-FAE. A new distribution of feruloyl esterases based on descriptor-based classification and structural analysis resulted in 13 families [64, 65], which are now distributed in ESTHER as SF1,2,3,4,9,10,11 in Tannase, SF5 in FaeC, SF6 Esterase_PhB (CE-1 in the subdivision of CaZy [66]), SF7 Lipase_3, SF8 A85_Feruloyl-esterase, SF12,13 in Fungal_Carb_Lip.

Alpha/beta hydrolases involved in plastic synthesis and degradation of petroleum-based plastics

Industrial needs justify biotechnological applications of enzymes instead of harsh chemical procedures, a feature which led to numerous reports of “novel” enzymes selected for their improved catalytic activities: e. g., lipases for laundry, esterases for biodiesel production. Eukaryotic lipases working at water-lipid interfaces of micelles are able to hydrolyze complex polymers such as self-emulsifying lipid excipients used to improve the oral bioavailability of water-insoluble drugs [67]. Therefore, it is not surprising to find related enzymes acting on more solid substrates, and

optimizing the reaction conditions to increase their efficiency and selectivity for the more recalcitrant forms of plastics commonly found in waste is a promising strategy [68]. The recent discovery of microorganisms able to destroy plastics led to numerous pieces of work aimed at improving enzymes, to make them able to both reduce the overwhelming load of plastic waste and depolymerize it back into its (monomeric) precursors, as a strategy for recycling them without using even more fossil carbon sources [69]. It is not surprising that some enzymes acting on natural polymers evolved naturally to degrade man-made plastics. Some alpha/beta hydrolases only hydrolyze ester bounds in plastics. For example, true polyurethanases are members of the Amidase signature (AS) superfamily instead of being alpha/beta hydrolases [70], but urethane polyesters are substrates for some members of the alpha/beta hydrolase fold superfamily [71]. Nylon esterase is not an alpha/beta hydrolase but a 6-aminohexanoate-oligomer endohydrolase. Polyethylene terephthalate is the most common thermoplastic polymer resin of the polyester family and many enzymes of the Polyesterase-lipase-cutinase family are able to deconstruct them (<https://bioweb.supagro.inrae.fr/ESTHER/substrate?name=Polyethylene-terephthalate&class=Substrate>). Some enzymes reach very high capacity to efficiently degrade amorphous polyethylene terephthalate [72]. Nowadays the search for new enzymes with thermotolerant and improved activities is intensive [73].

Multi-target inhibitors

Dual or multi-target drugs have raised considerable interest lately owing to their advantages in the treatment of complex diseases such as Alzheimer's disease (AD) [74]. For a long time, the only approved treatment alleviating the AD symptoms of acetylcholine deficit have been acetylcholinesterase inhibitors, but the sister enzyme butyrylcholinesterase is now considered as another suitable target together with acetylcholinesterase. Many other pathways leading to AD could also be targeted, and combining multiple pharmacophores in one single drug molecule is now an active field of research. Beside cholinesterase inhibition, the desired activities include: antioxidant properties, anti-amyloid beta-aggregation, anti-tau-aggregation, GSK-3 kinase inhibition, monoamine-oxidase inhibition, cannabinoid-receptor ligand, FAAH inhibition, calcium channel antagonism, 5-HT-receptor ligand, histamine H3 receptor, muscarinic antagonism, GABA-transporter inhibition. A view on the diversity of compounds is provided on ESTHER page https://bioweb.supagro.inrae.fr/ESTHER/typinhib?name=Multitarget&class=Type_inhibitor.

Other human alpha/beta hydrolases have also been selected for dual inhibition. Epoxide hydrolase inhibitors are coupled to pharmacophores inducing LTA4-hydrolase inhibition, 5-lipoxygenase inhibition, PPARgamma ligand or NMDA ligand activities to treat cancerous cells. Monoglyceride lipase/lysophospholipase inhibitors are coupled to FAAH inhibitors.

Heterophilic protein-protein interactions triggered by alpha/beta hydrolases

An increasing number of members of the superfamily functionally depend on interactions with specific partners. As these interactions do not involve the core of the fold, the addition of bundles, lids, internal subdomains or N- or C-terminal extensions resulting from exon shuffling or mRNA alternative splicing are key elements of the linkage, and they are not conserved across families. The following table (Table 3) summarizes the most prominent examples described so far. These data have been proven experimentally. They do not rely on data extracted from protein-protein interaction databases which not surprisingly provide many more hits, some of which might be of utmost importance in the future.

Table 3 Families where members have functional protein partners.

(Homophilic interactions and enzymes acting on protein substrates or ubiquitin-degradation pathways are not included.)

Representative family	Protein partner
ABHD6-Lip	Lipase-specific foldase LifPc; GluA1 receptor
abh_upf0017	ABHD15 phosphodiesterase 3B PDE3B
Acetylcholinesterase (ACHE)	ColQ aka collagenic tail peptide, AChE Q subunit, acetylcholinesterase-associated collagen; beta-amyloid peptide
Arb2_FAM172A	Ago2 (Argonaute-2); Chd7

Asp2	Serine-rich repeat glycoprotein GspB
Bacterial lip FamI.1	Lipase foldase
Bacterial lip FamI.2	Lipase chaperone LimA lifo
Butyrylcholinesterase (BCHE)	ColQ (see above)
Carboxypeptidase S10	Beta-galactosidase; neuraminidase
Carb B Bacteria	Chaperon
Carb B Chordata	Beta-glucuronidase
CGI-58 ABHD5 ABHD4	Adipocyte triglyceride lipase (ATGL or PNPLA2); PNPLA9(PLA2G6)
Cholesterol esterase	Grp 94 chaperone-related protein p94
CIB-CCG1-interacting-factor-B	Transcription initiation factor TFIID
DPP4N Peptidase S9	Kv 4.2 channels
Duf 726	Binds Apm2 and contributes to its membrane recruitment
Glilotactin	Discs large; anaconda
Glutactin	Yet unknown
Fungal carboxylesterase lipase	VdtD Dirigent Protein of the multicopper oxidase VdtB
Hydrolase RBBP9 YdeN	Retinoblastoma-binding protein 9
Lipase 3	Protein disulfide-isomerase PDI, lipase-specific foldase Lif
Lipoprotein_Lipase	Glycosylphosphatidylinositol-anchored high-density; lipoprotein-binding protein 1 (GPIHBP1); angiopoietin-like protein 4 (ANGPTL4); apolipoprotein C2 (APOC2)
Masparidin aka ACP33, SPG21_like, GL010 protein, MASTBM-019 protein	Intracellular domain of CD4; aldehyde dehydrogenase ALDH16A1
Neurologin	Neurexins; meprin, A-5 protein, and receptor protein-tyrosine phosphatase mu [MAM] domain-containing glycosylphosphatidylinositol anchor (MDGA); protein-tyrosine phosphatase receptor type1 (PTPRT); thrombospondin-1 (TSP1); NMDA receptor subunit GluN1; hevin aka SPARCL1; MADD-4 aka punctin [75]
Neurotactin	Amalgam
NLS3-TeX30	Microtubule-associated proteins
Pancreatic lipase	Colipase
Plant lipase EDS1-like	EDS1 interacts with PAD4 or SAG101
RsbQ-like	Dwarf53 (D53); D3 More Axillary Growth2 (MAX2)
T6SS-TLE3	VgrGAIEC C-terminus adaptor
Thyroglobulin CHEL domain	Other TG domains; autoimmune antibodies

Future direction

ESTHER is a knowledge-based resource that aims to link the sequences, specificities and structural features of proteins or protein domains with an alpha/beta hydrolase fold. When these proteins are enzymes, detailed structure-function analysis for well-characterized reactions is key to predict the substrate specificity of related members. However, for many sub-families there is still a long way to go before reliable automated substrate prediction is available. Many of the new techniques described above will pave the way to a better understanding of the place of these enzymes in diverse metabolic pathways.

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